Adapter-directed display systems

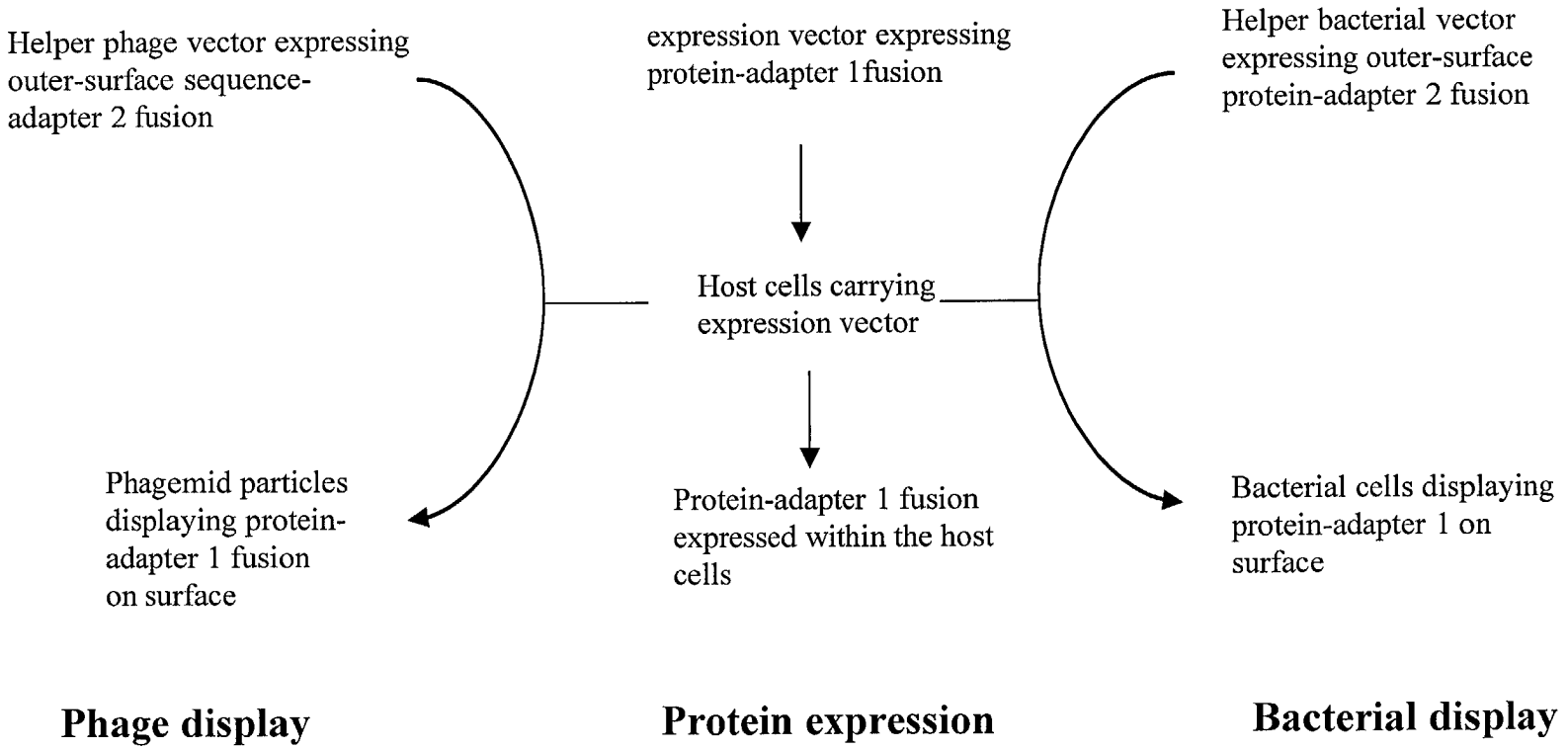


Fig. 1

KO7kpn phage Screening by ELISA

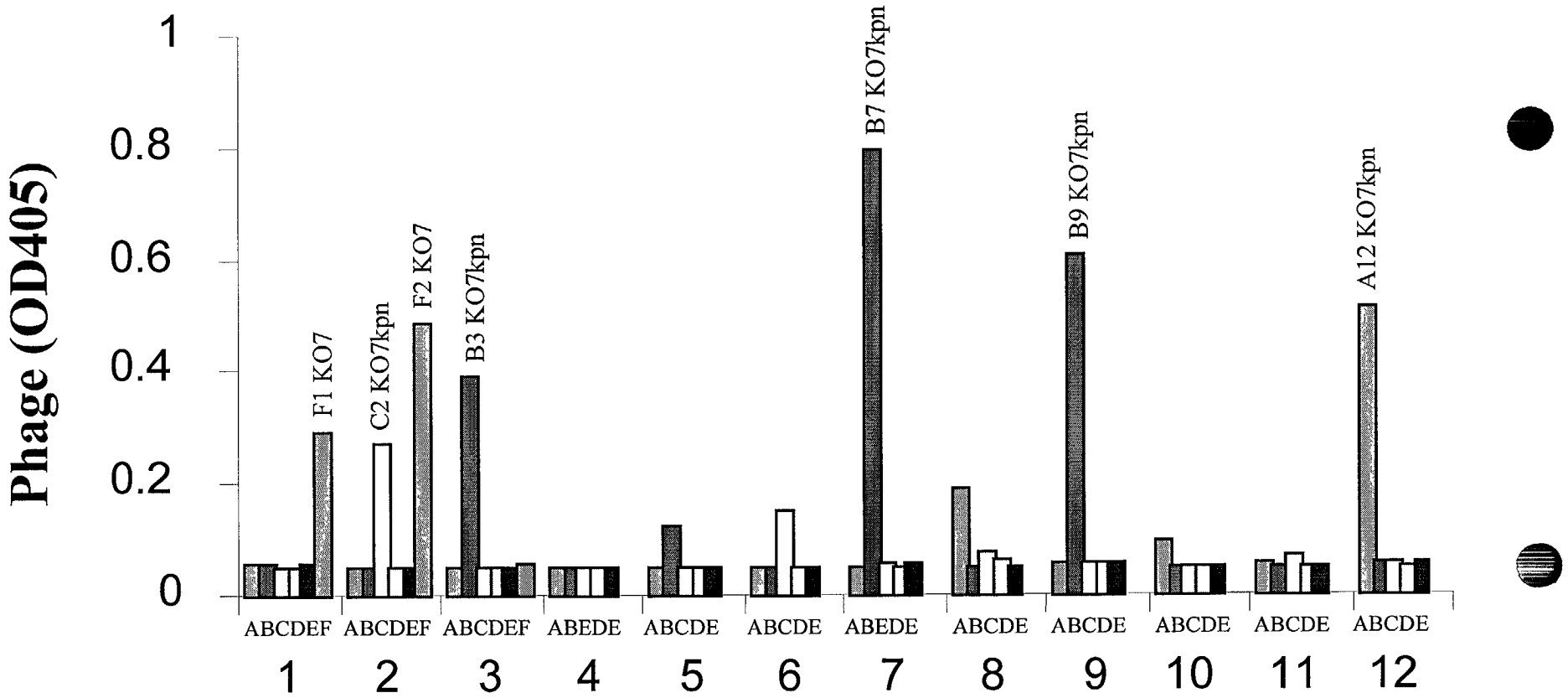


Fig. 2

KO7kpn helper phage Vector

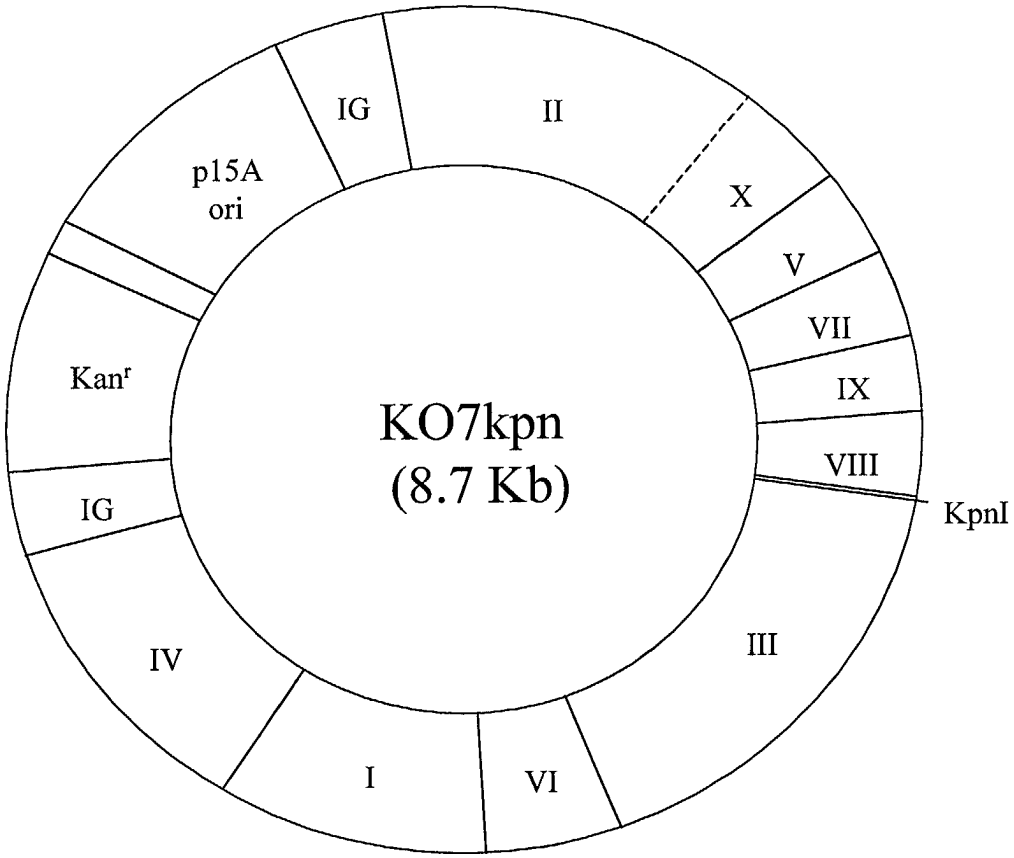


Fig. 3A

Gene III leader sequence in KO7 helper phage

GTG	AAA	AAA	TTA	TTA	TTC	GCA	ATT	CCT	TTA	GTT	GTT	CCT	TTC	TAT	TCT	CAC	TCC	GCT
V	K	K	L	L	F	A	I	P	L	V	V	P	F	Y	S	H	S	A

Gene III leader sequence in KO7kpn helper phage

KpnI

GTG	AAA	AAA	TTA	TTA	TTC	GCA	ATT	CCT	TTA	GTG GTA CCT	TTC	TAT	TCT	CAC	TCC	GCT
V	K	K	L	L	F	A	I	P	L	V	V	P	F	Y	S	H	S	A

Fig. 3B

Map of phagemid vector pABMC6

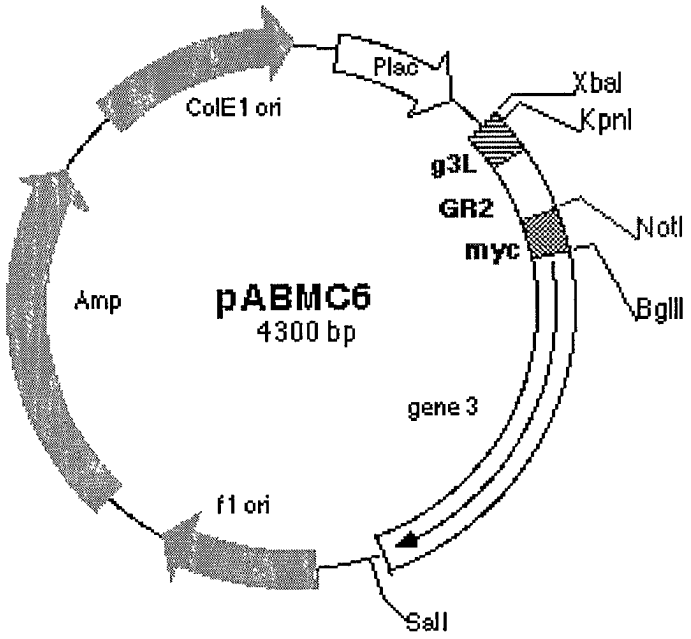


Fig. 4

Helper phage with engineered gene III fused to adaptor 2

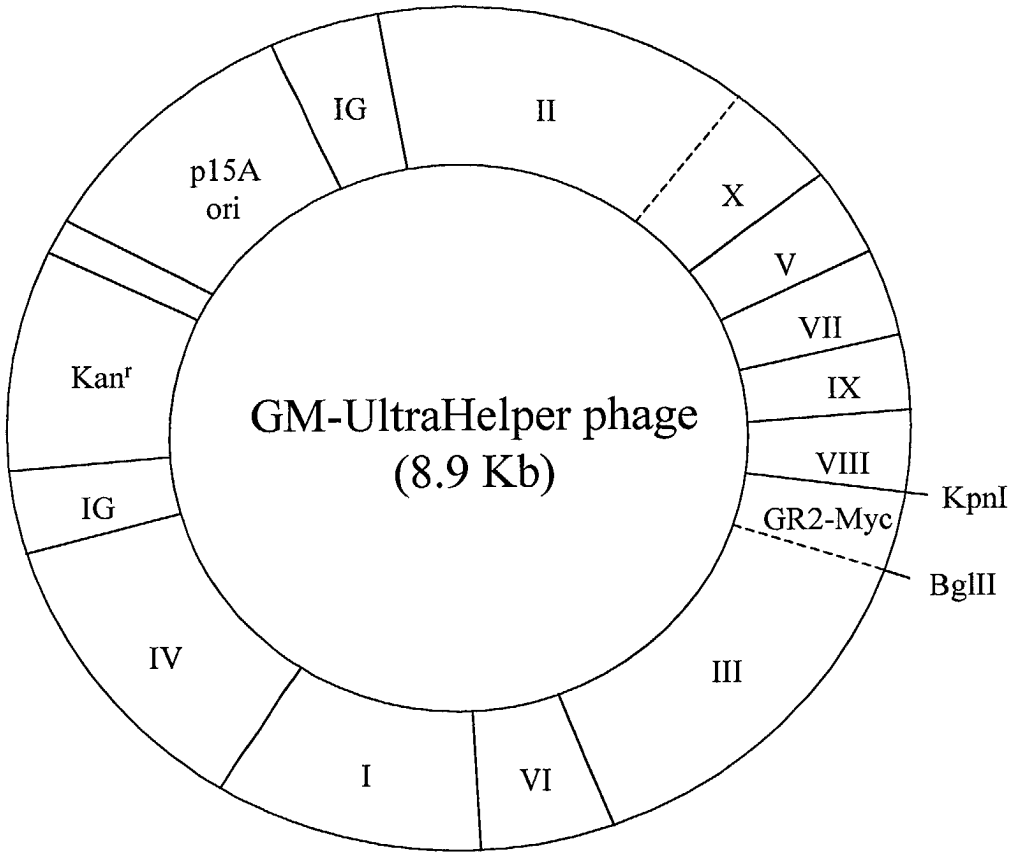


Fig. 5A

GR2-Myc domain coding sequence in GM-UltraHelper phage genome

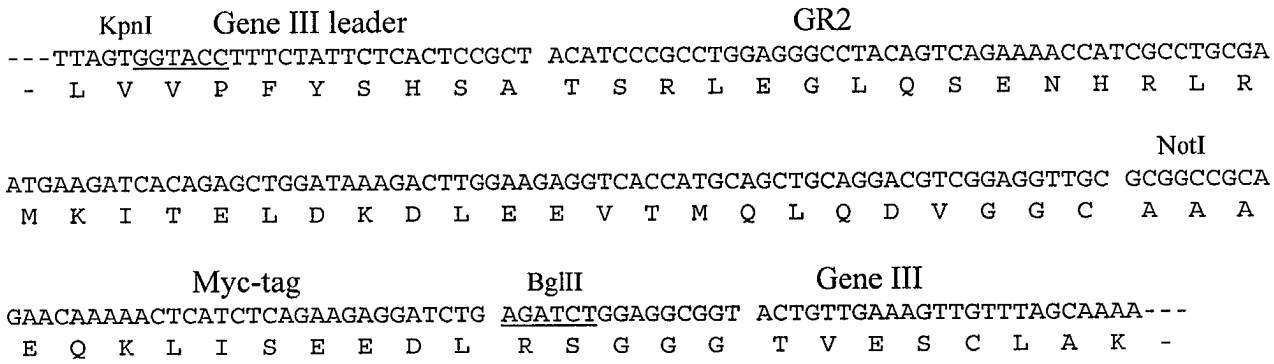


Fig. 5B

Trypsin cleavage sites at GR2-Myc domain on GM-UltraHelper phage

GR2 domain

T S R▲L E G L Q S E N H R▲L R▲M K▲I T E L D K▲D L E E V

Myc-tag

T M Q L Q D V G G C A A A *E Q K▲L I S E E D L R▲S* G G G

Fig. 5C

GR2-Myc-pIII fusions assembly into GM phage particles

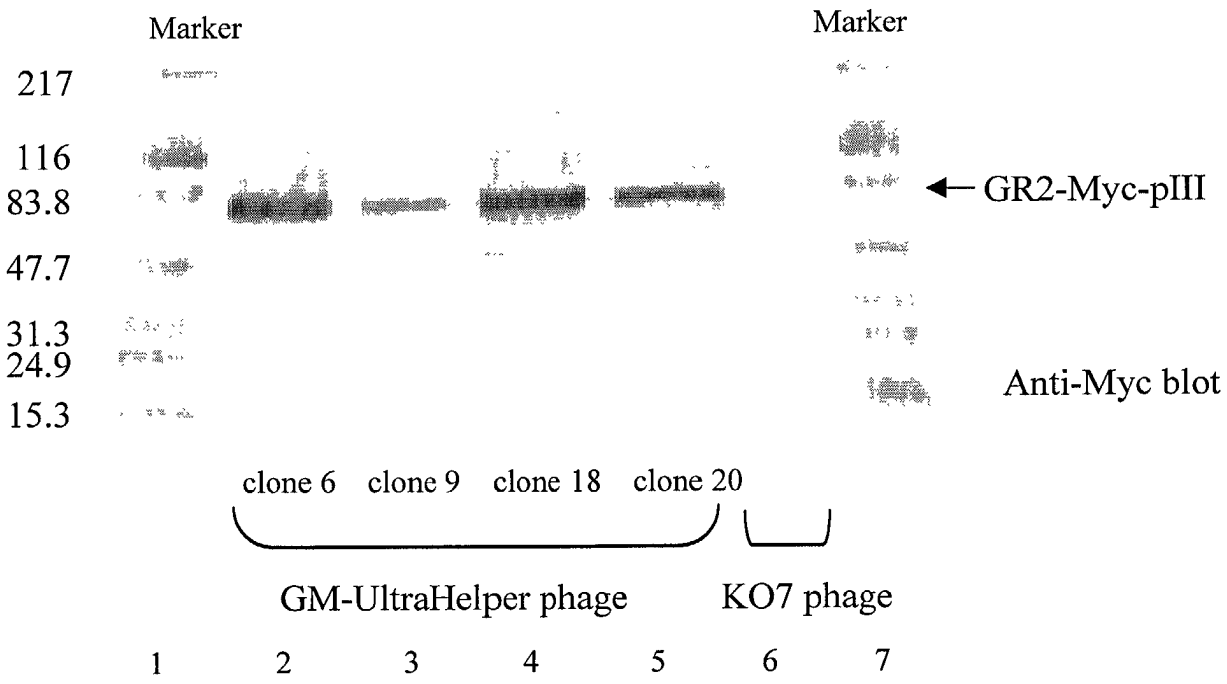


Fig. 6

Detection of GR2-Myc domain on GM-UltraHelper phage

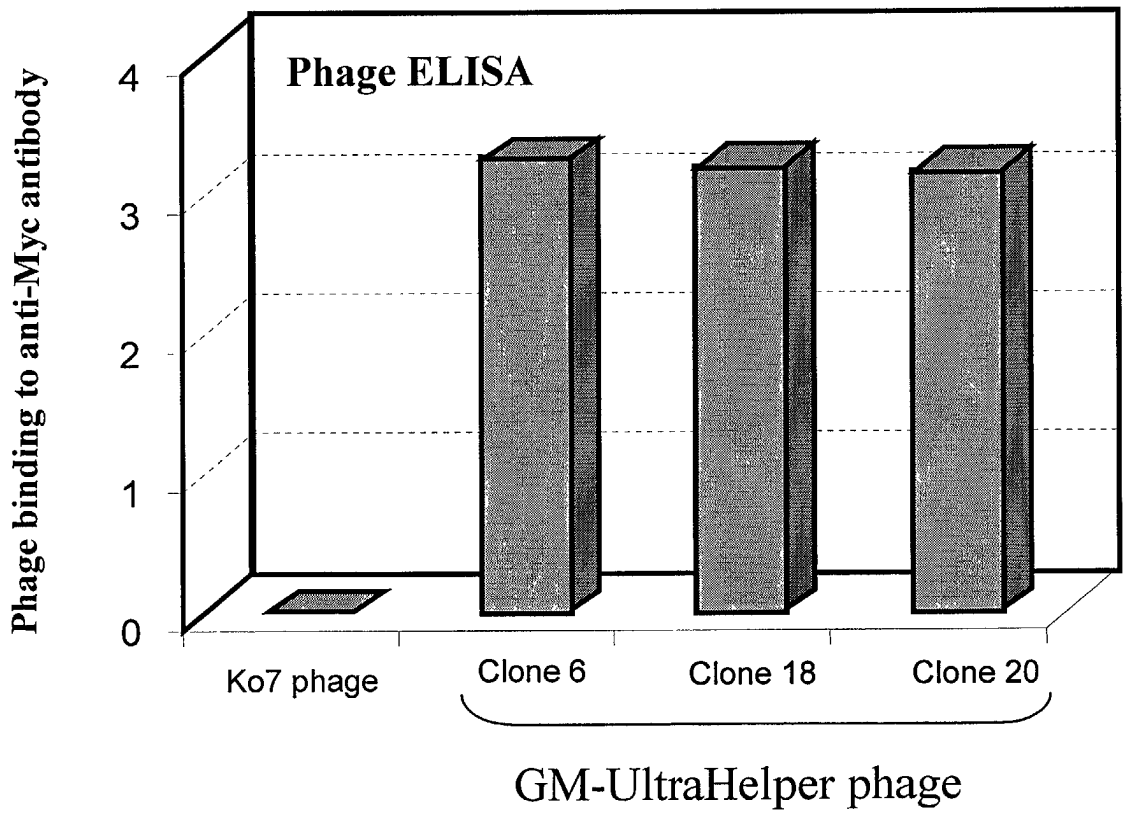
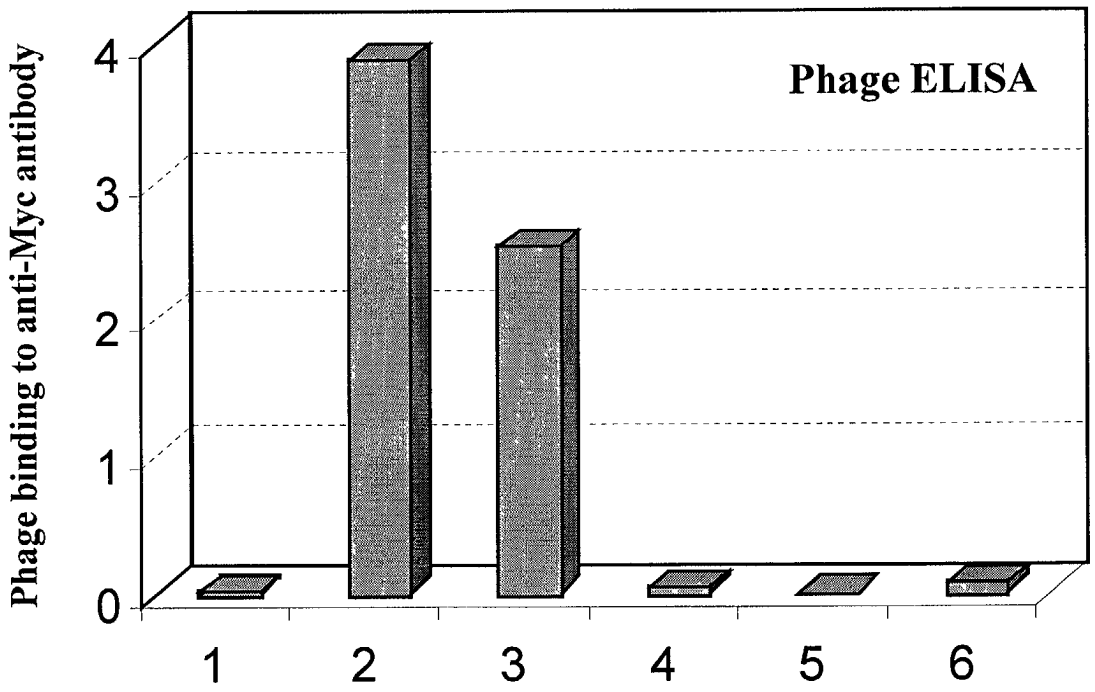


Fig. 7

Cleavage of GR2-Myc domains on GM phages by trypsin



1) KO7 phage
2) GM phage
3) GM phage + 1ug/ml Tryp
4) GM phage + 5ug/ml Tryp
5) GM phage + 10ug/ml Tryp
6) GM phage + 50ug/ml Tryp

Fig. 8

Phagemid vector for protein-GR1 expression

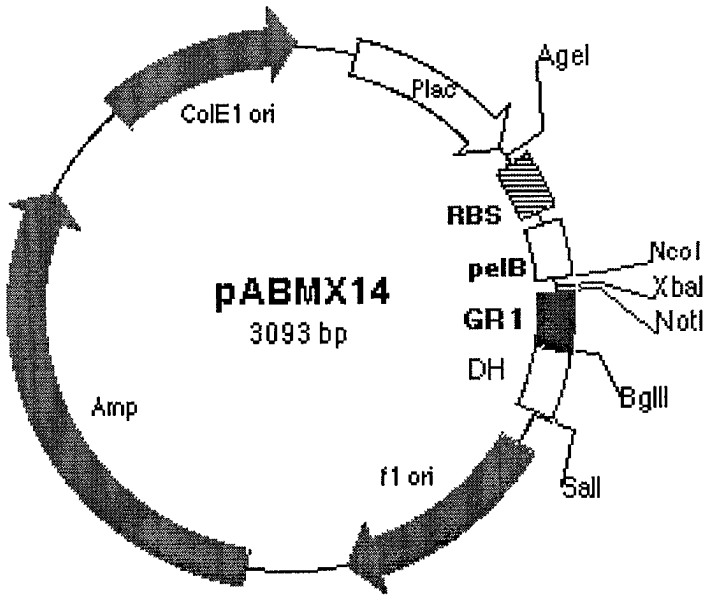


Fig. 9A

Complete vector sequence of pABMX14

GCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACCGGTTCTTTAAGGAGGA
ATTAAAAAATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCGGCCCTGCAGGCCTCTAGAGCGGCCGCTGGAGGTGAGGAGAAGTCCCGGCTG
TTGGAGAAGGAGAACCGTGAACTGGAAAAGATCATTGCTGAGAAAGAGGAGCGTGTCTCTGAACTGCGCCATCAACTCCAGTCTGTAGGAGGTTGTAGATCTTATCCATACGACGTACCAGACTA
CGCAGGAGGTCATCACCATCATCACCATTAATGAGTCGACCTCGACCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTT
ACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAG
CGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCC
GTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTT
CGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTG
GTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAAT
ACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTT
GCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGC
CCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGT
TGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGAC
CGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATG
GCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGG
CTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTA
TGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGG
ATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG
CGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTG
TCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGG
TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATG
AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCG
GGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCAC
ATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA
GCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA

Fig. 9B

Functional display of scFv by GM-UltraHelper phage

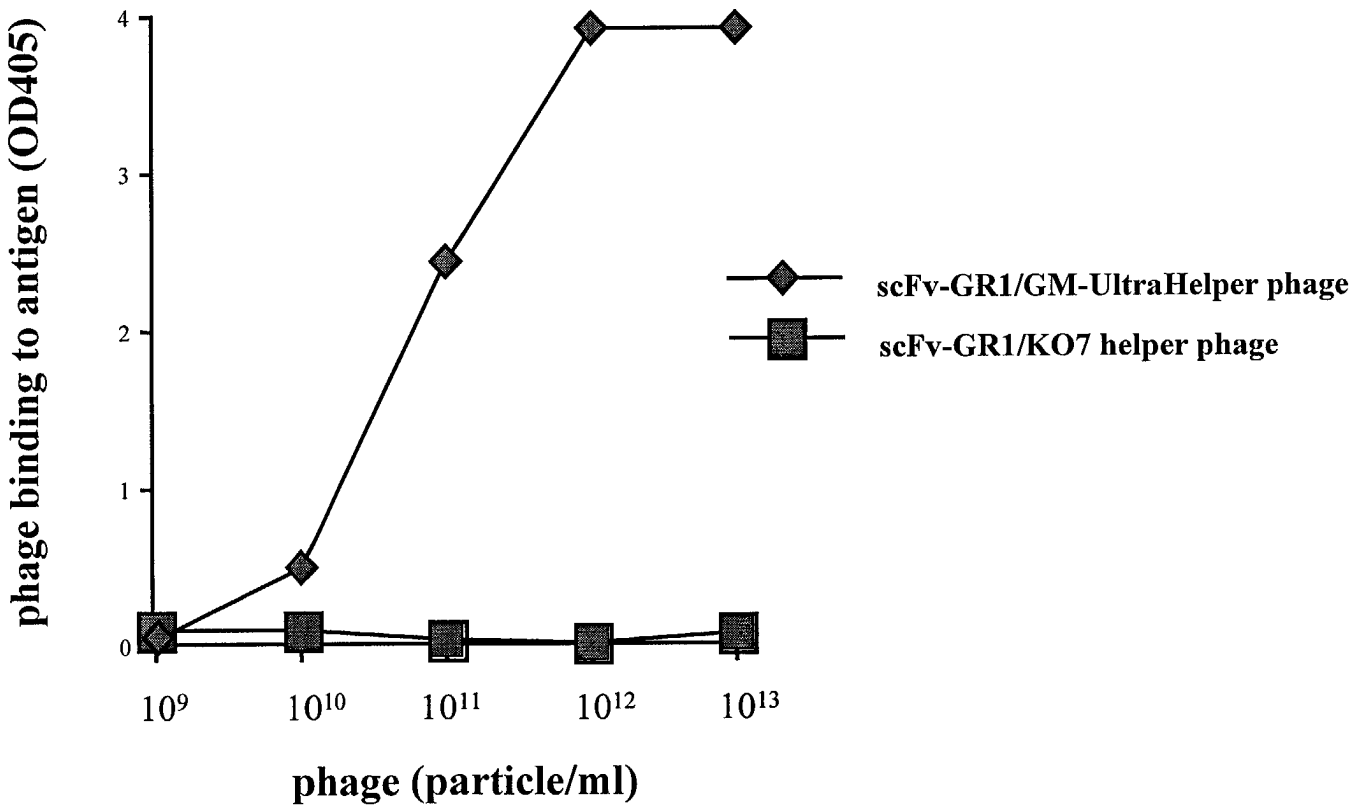


Fig. 10

Mutivalent display of scFv by GM-UltraHelper phage

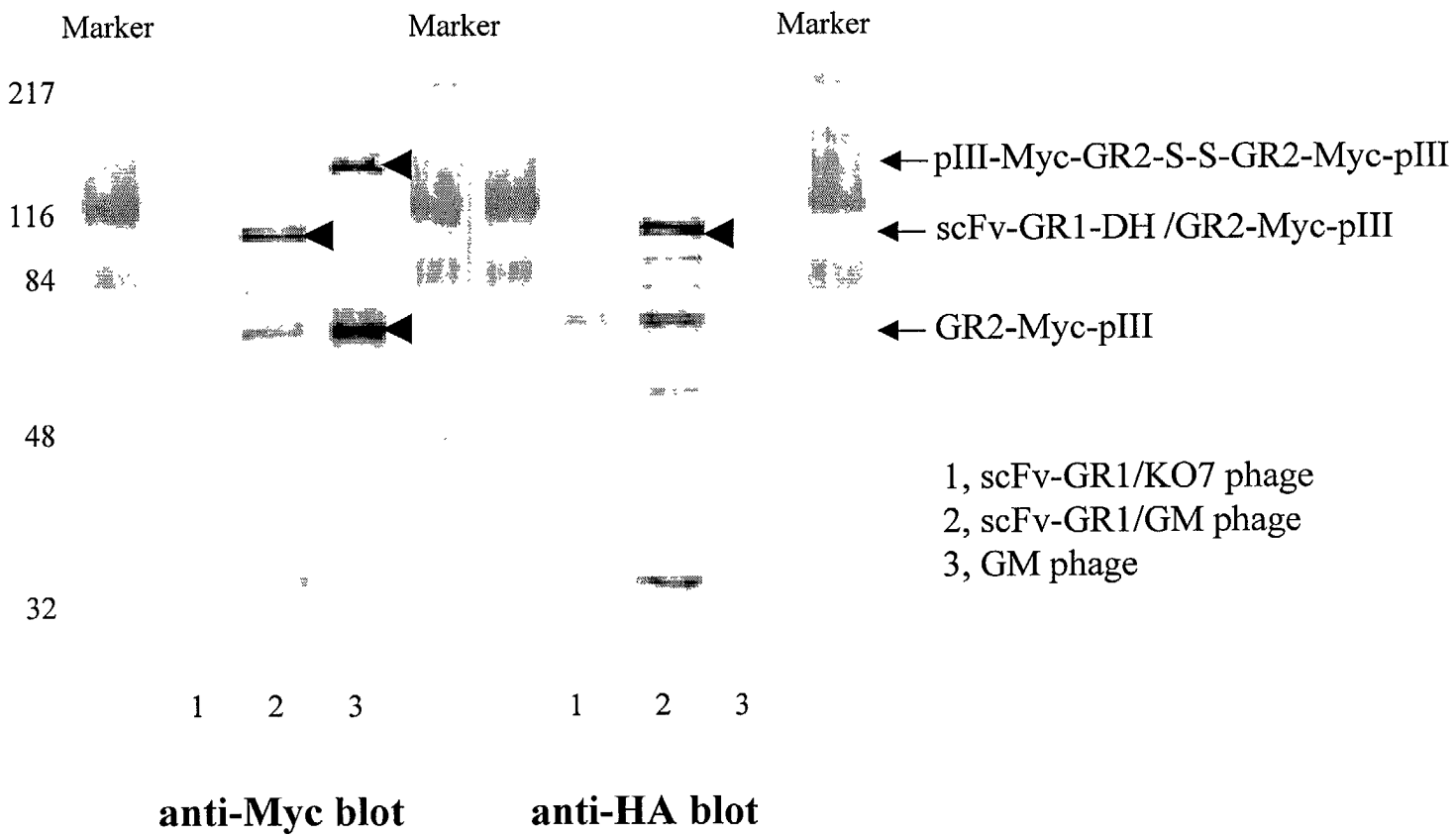


Fig. 11

Map of phagemid vector pABMC13

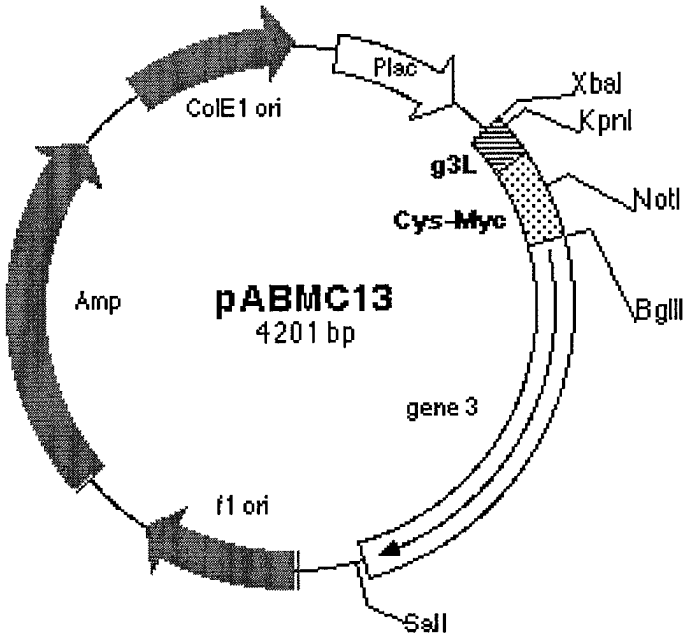


Fig. 12

Helper phage with Cys-Myc-pIII fusion gene

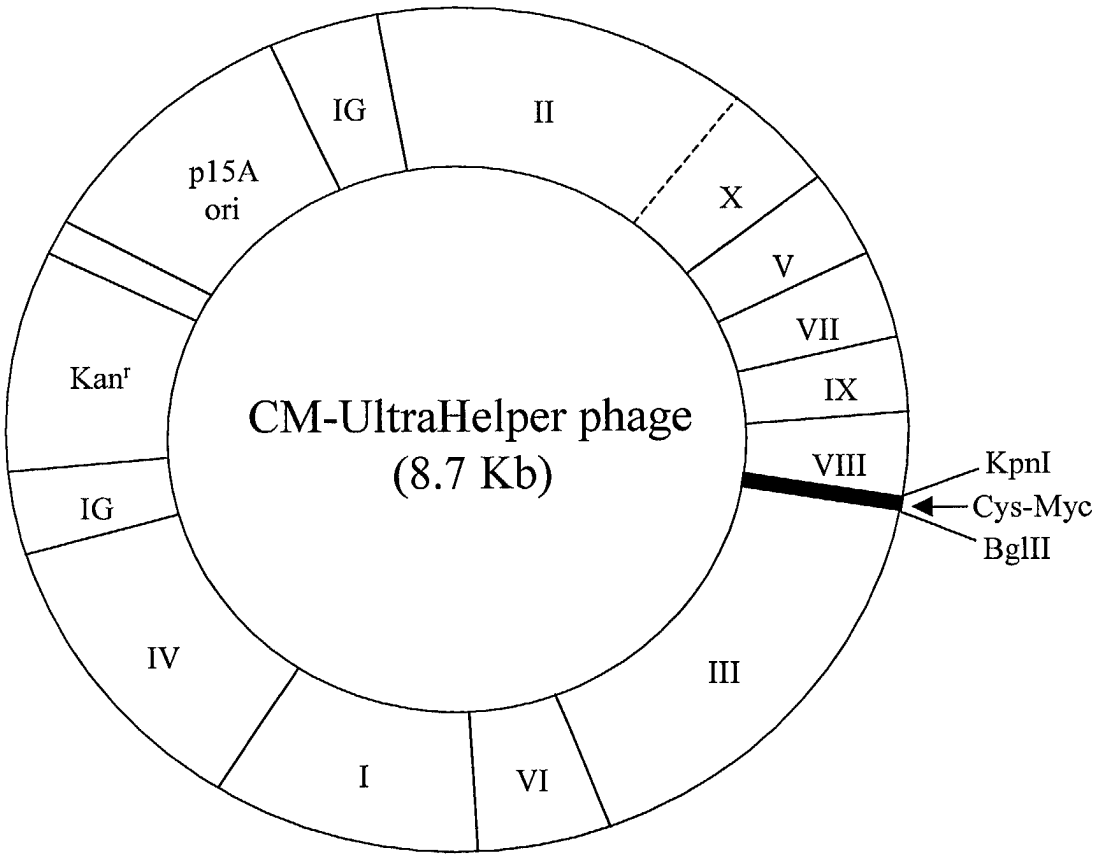


Fig. 13A

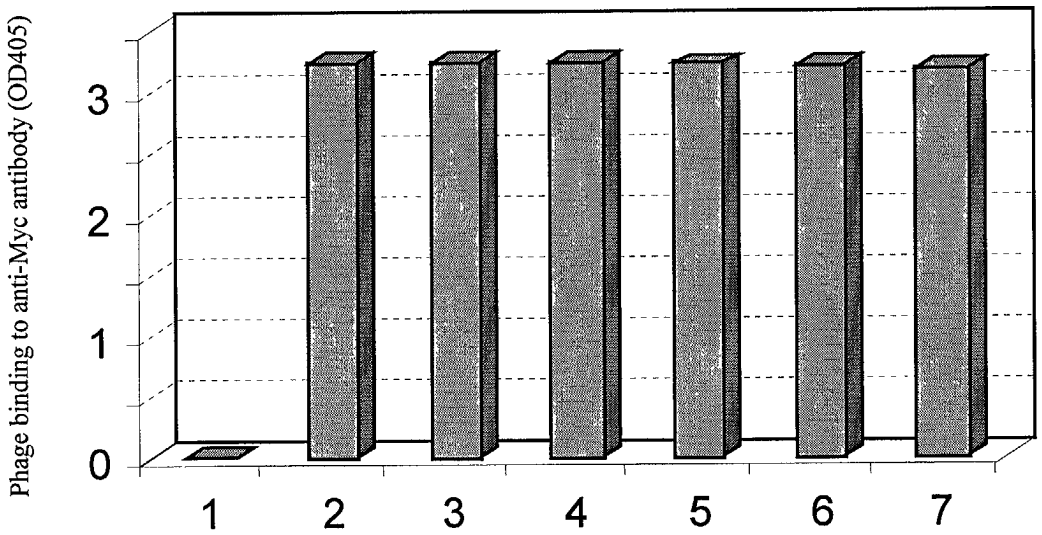
Engineered gene III sequence in CM phage

```
          KpnI  Gene III leader   Amber stop        NotI                Myc-tag                BglII
---TTAGTGGTACCTTTCTATTCTCACTCCGCT TAGGCTTGCGGTGGTGCGGCCGCAGAACAAAAACTCATCTCAGAAGAGGATCTGAGATCT AGATCTGGA
 -  L  V  V  P  F  Y  S  H  S  A  *  A  C  G  G  A  A  A  E  Q  K  L  I  S  E  E  D  L  R  S   R  S  G

        Gene III
GGCGGT ACTGTTGAAAGTTGTTTAGCAAAACCTCATACAGAAAATTCATTTACTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTACGCT------
 G  G   T  V  E  S  C  L  A  K  P  H  T  E  N  S  F  T  N  V  W  K  D  D  K  T  L  D  R  Y  A  -  -
```

Fig. 13B

Detection of Myc-tag on CM-UltraHelper phages by ELISA



1, KO7 phage
2, CM phage clone A9
3, CM phage clone B6
4, CM phage clone B7
5, CM phage clone B8
6, CM phage clone B9
7, GM phage control

Fig. 14

Phagemid vector for protein-HA-cys expression

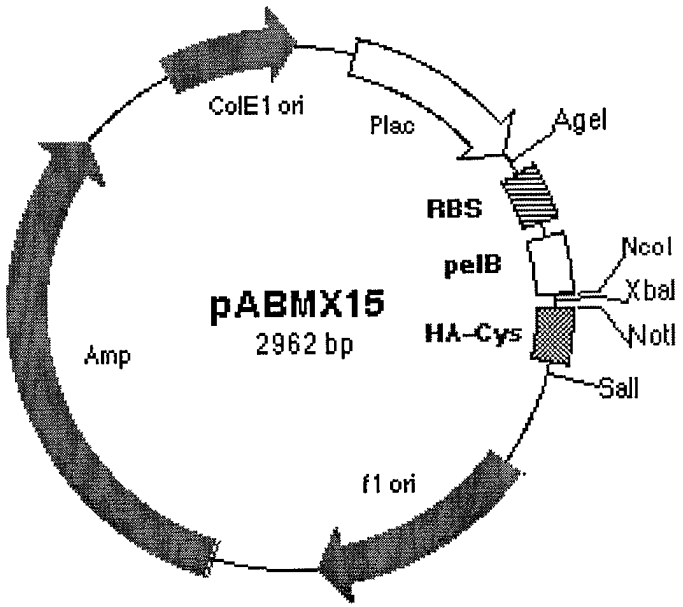


Fig. 15A

Complete vector sequence of pABMX15

GCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACCGGTTCTTTTAACTTTAGTAAGGAGGA
ATTAAAAAATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCGGCCCTGCAGGCCTCTAGAGCGGCCGCTTACCCGTACGACGTTCCGGACTACGCAGGTGGCT
GCTGATAAGTCGACCTCGACCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTC
GCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACC
GCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCAC
CTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTC
AACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGTGGCA
CTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAAC
ATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCA
ACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACT
ATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGA
TCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGG
CAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTG
CTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGA
TCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCA
TGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAG
CGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCAC
CGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGT
GCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAG
AGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACG
CGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCG
AGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA

Fig. 15B

Functional display of scFv by CM-UltraHelper phage

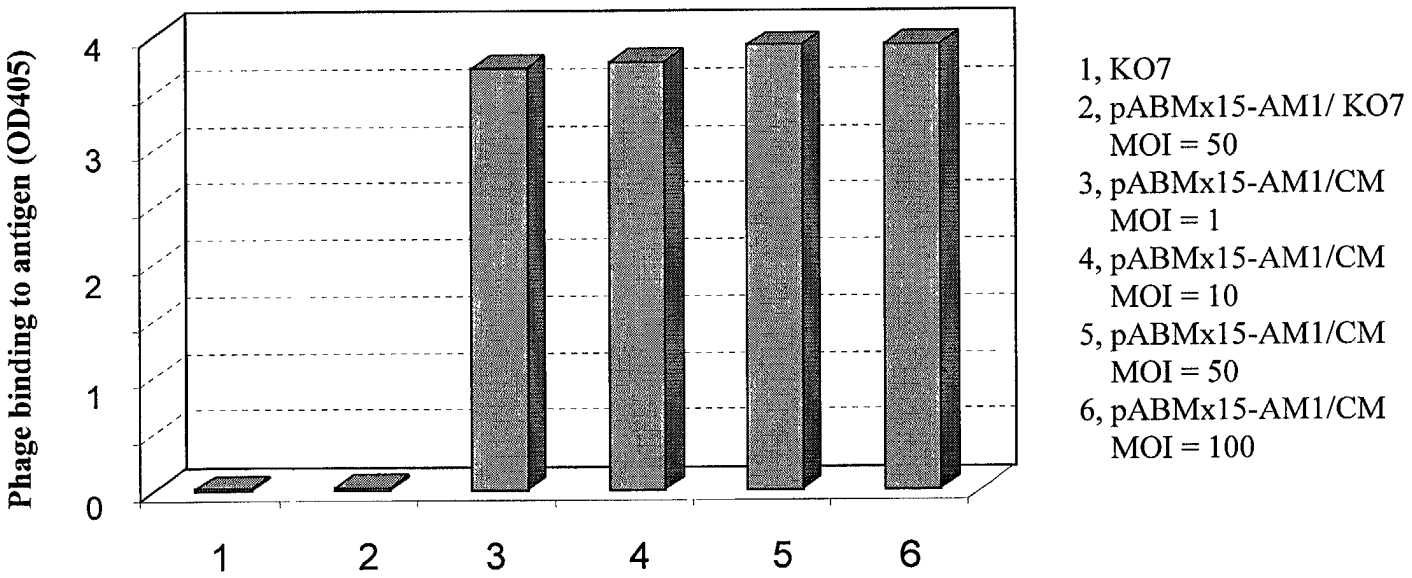
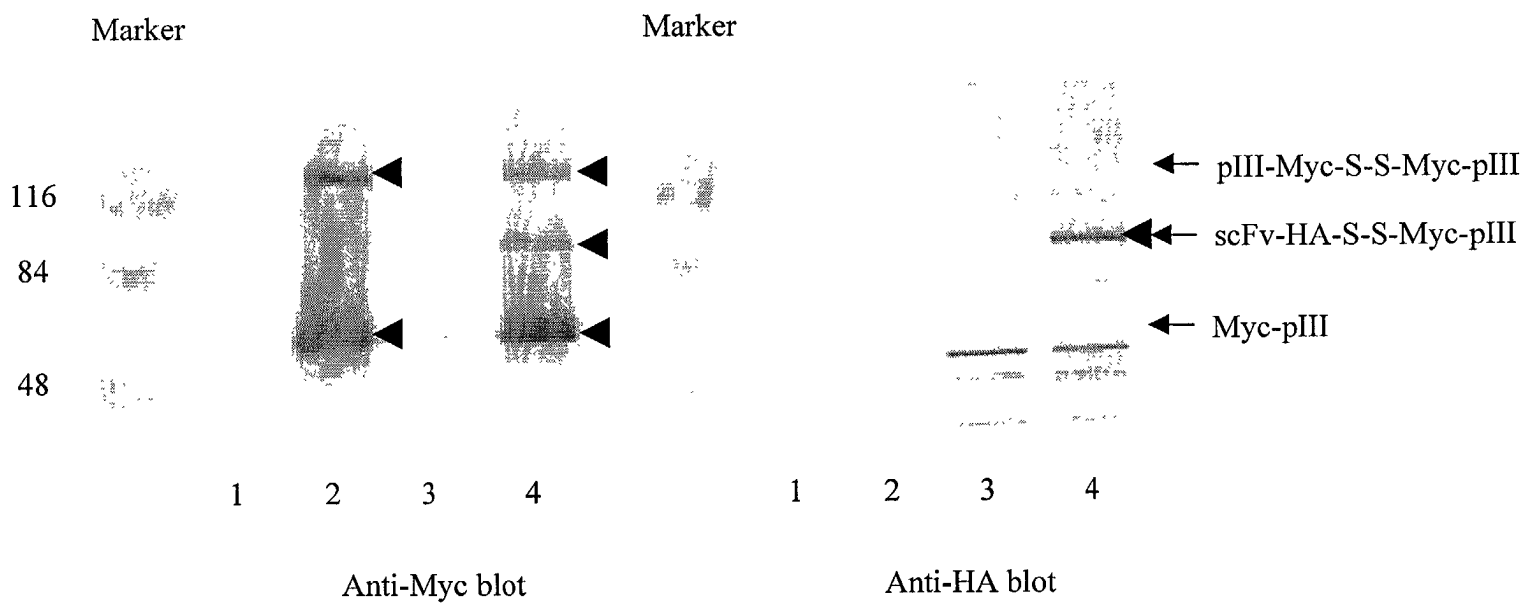


Fig. 16

Detection of scFv displayed by CM-UltraHelper phage



1: KO7 phage; 2: CM phage; 3: pABMx15-AM1/ KO7; 4: pABMx15-AM1/CM

Fig. 17

Map of phagemid vector pABMC12

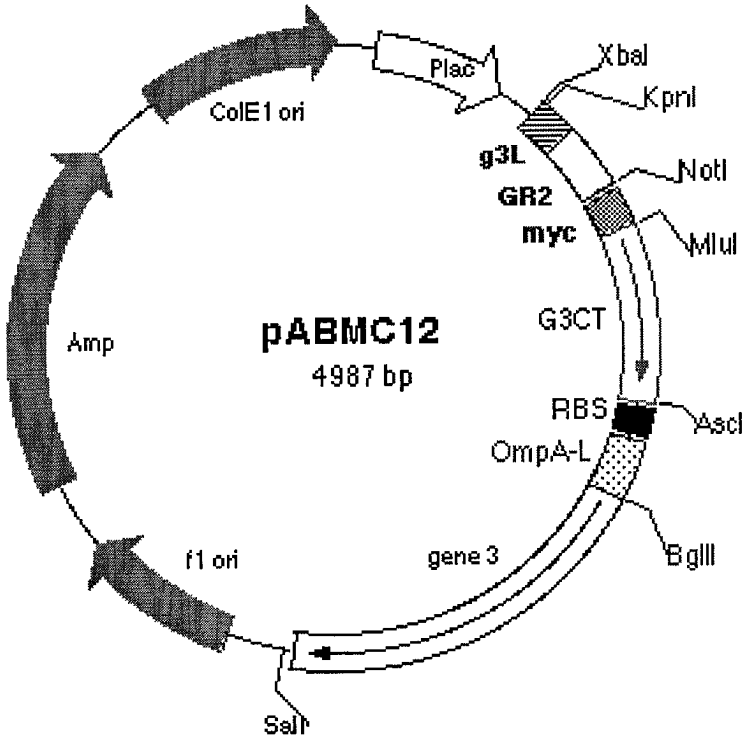


Fig. 18

Helper phage with an additional copy of engineered gene III

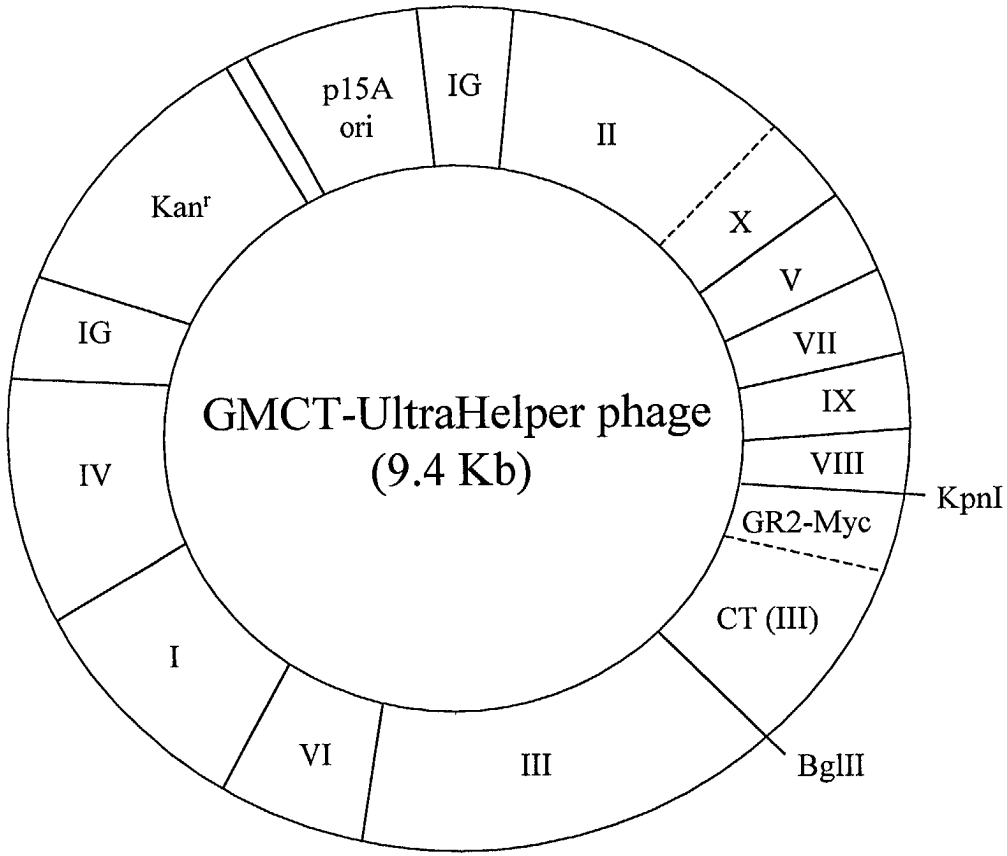


Fig. 19A

Engineered gene III Sequence in GMCT phage genome

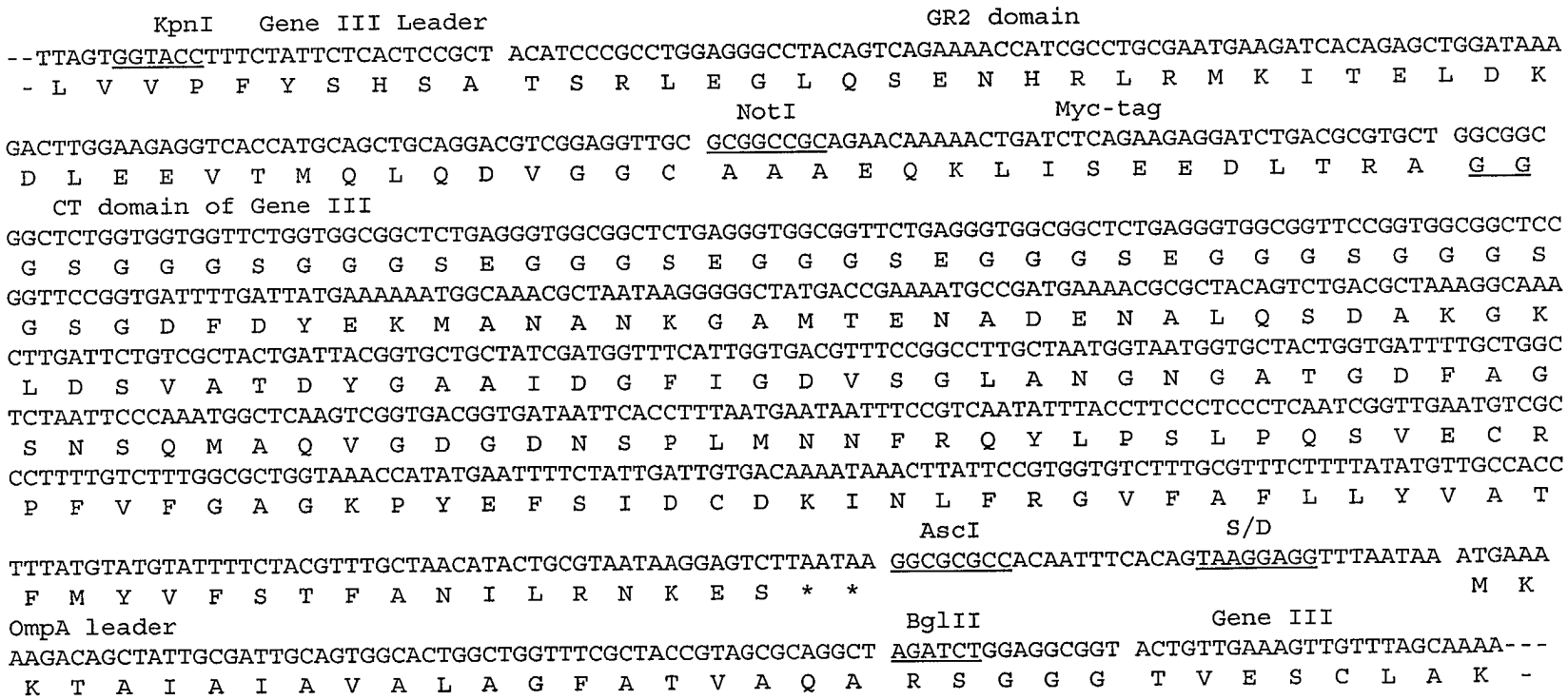
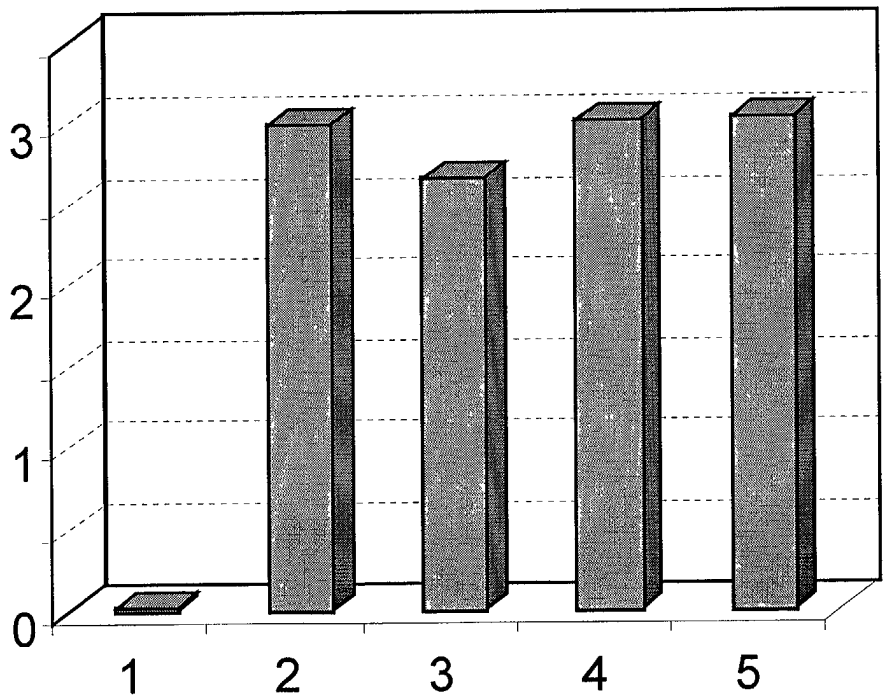


Fig. 19B

Functional display of scFv by GMCT-UltraHelper phage



1, pABMx14-AM1/ KO7
MOI = 50
2, pABMx14-AM1/GMCT
MOI = 1
3, pABMx14-AM1/GMCT
MOI = 10
4, pABMx15-AM1/GMCT
MOI = 50
5, pABMx14-AM1/GMCT
MOI = 100

Fig. 20

Detection of scFv displayed by GMCT-UltraHelper phage

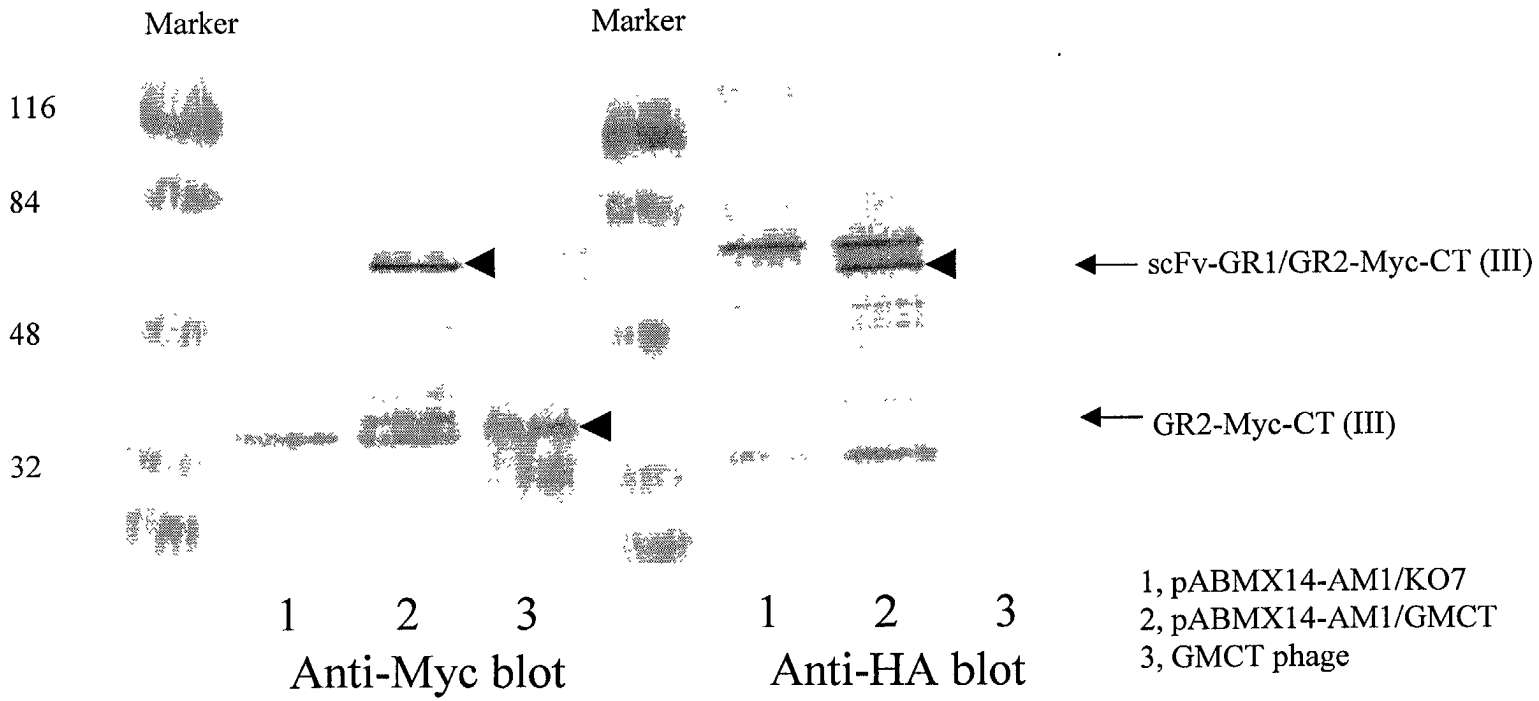


Fig. 21

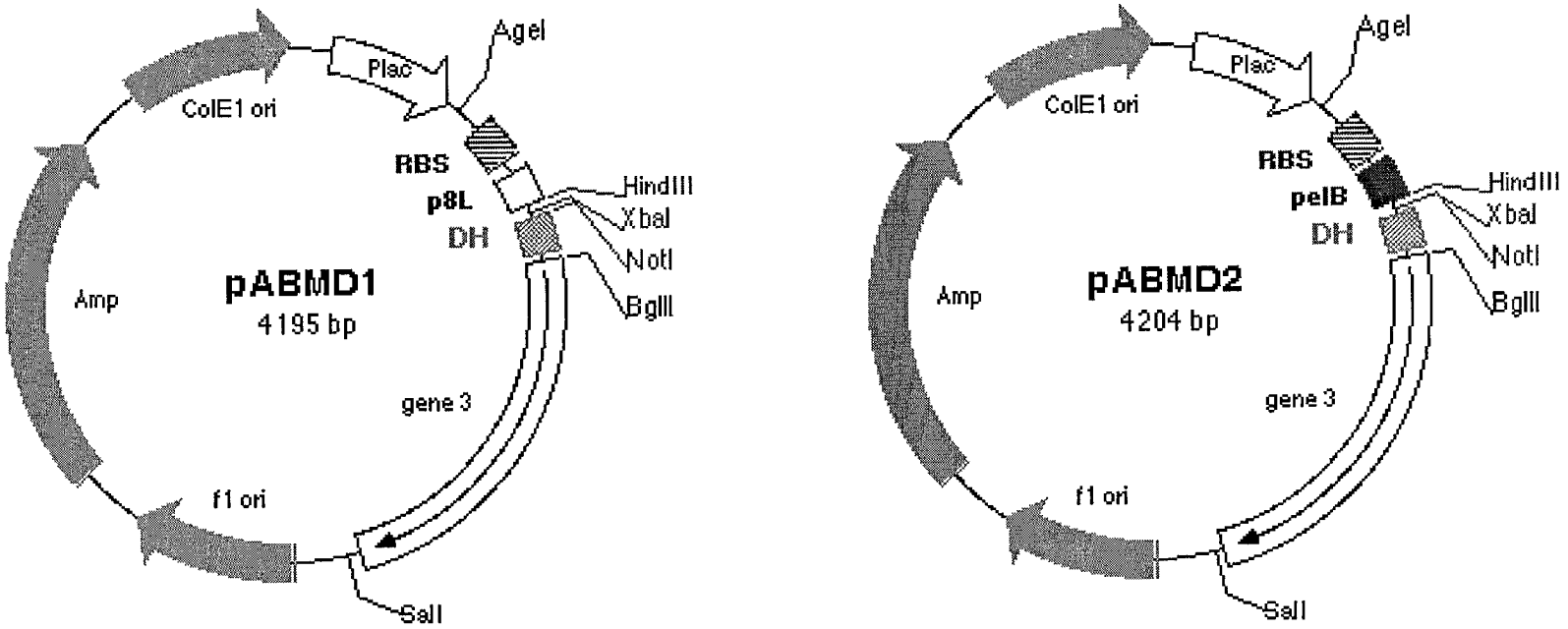

AgeI
Plac
ColE1 ori
RBS
p8L
DH
HindIII
XbaI
NotI
BglII
pABMD1
4195 bp
Amp
gene 3
f1 ori
SalI
AgeI
Plac
ColE1 ori
RBS
pelB
DH
HindIII
XbaI
NotI
BglII
pABMD2
4204 bp
Amp
gene 3
f1 ori
SalI

Fig. 22A

PABMD1 vector: sequence from AgeI to SalI

```
     lac promoter/lac O1          AgeI                EP        S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA
                P8 Leader                                                HindIII     XbaI
ATGAAAAAGTCTTTAGTCCTCAAAGCCTCCGTAGCCGTTGCTACCCTCGTTCCGATGCTAAGCTTCGCT TCTAGA
 M  K  K  S  L  V  L  K  A  S  V  A  V  A  T  L  V  P  M  L  S  F  A   S  R
    NotI                  HA-tag                                 His-tag      Amber stop  BglII
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT   TAG    AGATCT
 A  A  A   Y  P  Y  D  V  P  D  Y  A   G  G   H  H  H  H  H  H    *     R  S
                          Gene 3                                                 SalI
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC
 G  G  G   T  V  E  S  C  L  A  K  ----   A  N  I  L  R  N  K  E  S  *
```

PABMD2 vector: sequence from AgeI to SalI

```
     lac promoter/lac O1          AgeI                EP        S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA
                pelB Leader                                              Nco I       PstI      XbaI
ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCGGCCCTGCAGGCCTCTAGA
 M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L  A  A  Q  P  A  M  A  A  L  Q  A  S  R
    NotI                  HA-tag                                 His-tag      Amber stop  BglII
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT   TAG    AGATCT
 A  A  A   Y  P  Y  D  V  P  D  Y  A   G  G   H  H  H  H  H  H    *     R  S
                          Gene 3                                                 SalI
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC
 G  G  G   T  V  E  S  C  L  A  K  ----   A  N  I  L  R  N  K  E  S  *
```

Fig. 22B

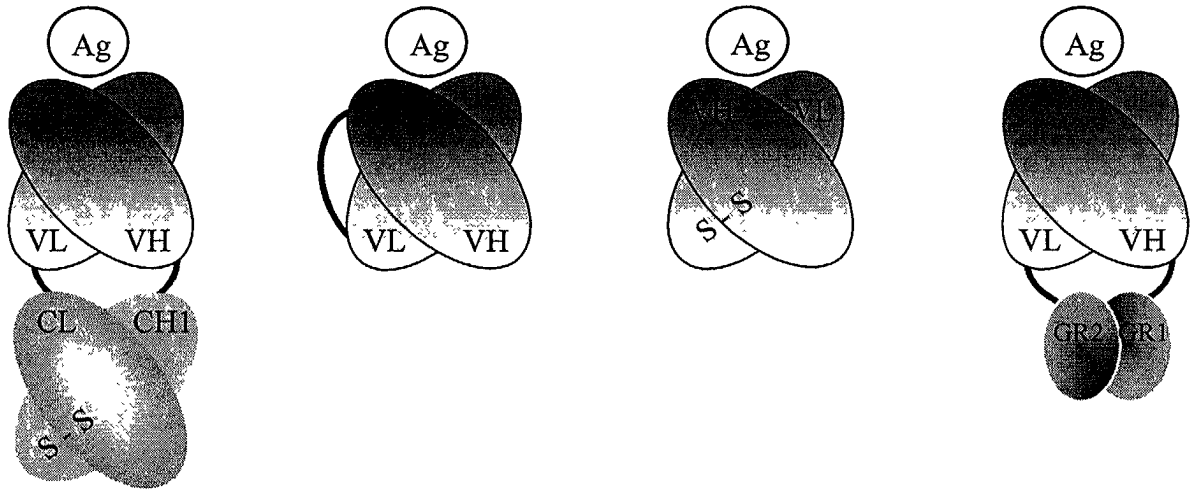
GR1 Sequence Range: 1 to 146

```
XbaI     10         20         30         40         50
TCTAGAGGTGGAGGAGGTGAGGAGAAGTCCCGGCTGTTGGAGAAGGAGAA
 S  R  G  G  G  G  E  E  K  S  R  L  L  E  K  E  N
         60         70         80         90        100
CCGTGAACTGGAAAAGATCATTGCTGAGAAAGAGGAGCGTGTCTCTGAAC
  R  E  L  E  K  I  I  A  E  K  E  E  R  V  S  E
       110        120        130        140  AscI
TGCGCCATCAACTCCAGTCTGTAGGAGGTTGTTAATAGGGCGCGCC
L  R  H  Q  L  Q  S  V  G  G  C  *  *
```

GR2 Sequence Range: 1 to 140

```
 XhoI    10         20         30         40         50
TCTCGAGGAGGTGGTGGAACATCCCGCCTGGAGGGCCTACAGTCAGAAAA
 S  R  G  G  G  G  T  S  R  L  E  G  L  Q  S  E  N
         60         70         80         90        100
CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAGG
  H  R  L  R  M  K  I  T  E  L  D  K  D  L  E  E
       110        120        130  NotI 140
TCACCATGCAGCTGCAGGACGTCGGAGGTTGCGCGGCCGC
V  T  M  Q  L  Q  D  V  G  G  C  A  A  A
```

Fig. 23



Fab

scFv
single-chain Fv

dsFv
disulfide- stabilized Fv

ccFv
heterodimeric coiled-coil stabilized Fv

Ag antigen

Fig. 24

Expression vector for Adapter-directed bacterial display

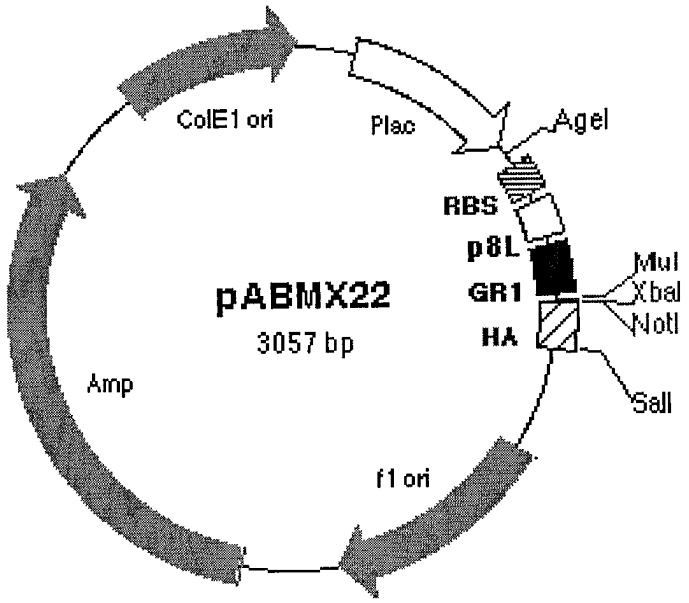


Fig. 25A

Complete vector sequence of pABMX22

GCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACCGGTTCTTTAAGGAGGAATT
AAAAAATGAAAAAGTCTTTAGTCCTCAAAGCCTCCGTAGCCGTTGCTACCCTCGTTCCGATGCTAAGCTTCGCTGGTGAGGAAAAGTCCCGTCTGCTGGAGAAAGAGAACCGTGAACTGGAAAAGATC
ATTGCTGAGAAAGAGGAGCGTGTTTCTGAACTGCGCCATCAACTGCAGTCTGTAGGCGGTTGCACGCGTTCTAGAGCGGCCGCTTACCCGTACGACGTTCCGGACTACGCATGATAAGTCGACCTCGA
CCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGT
AATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTAC
ACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGC
ACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGA
ACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCT
TACAATTTAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAA
AAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTG
CACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATT
GACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCAT
AACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGA
ATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAG
GCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCC
CTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATA
TACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAA
AAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGT
AACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTG
CTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACC
GAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAA
CGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGG
CCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCA
GTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA

Fig. 25B

Helper vector for adapter-directed bacterial display

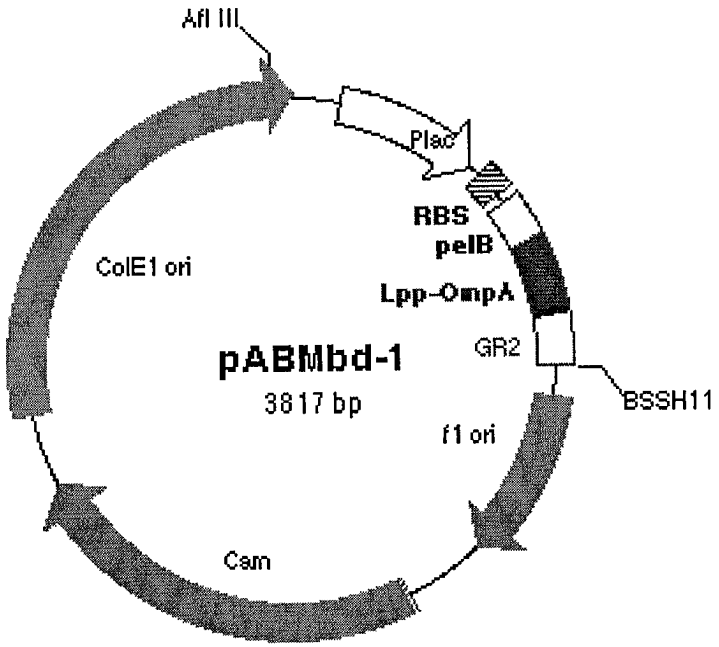


Fig. 26A

Complete vector sequence of pABMXbd-1

GCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCA
TGATTACGCCAAGCGCGTTTAACTTTAGTAAGGAGGAATTAAAAAATGAAATACCTGCTGCCGACCGCAGCCGCGGGTTTGCTGTTACTGGCGGCCCAGCCGGCTATGGCGATGAAAGCTACTAAACTG
GTACTGGGCAACCCGTATGTTGGCTTTGAAATGGGTTACGACTGGTTAGGTCGTATGCCGTACAAAGGCAGCGTTGAAAACGGTGCATACAAAGCTCAGGGCGTTCAACTGACCGCTAAACTGGGTTAC
CCAATCACTGACGACCTGGACATCTACACTCGTCTGGGTGGCATGGTATGGCGTGCAGACACTAAATCCAACGTTTATGGTAAAAACCACGACACCGGCGTTTCTCCGGTCTTCGCTGGCGGTGTTGAG
TACGCGATCACTCCTGAAATCGCTACCCGTCTGGAATACCAGTGGACGAACAACATCGGTGACGCACACACCATCGGCACTCGTCCGGACGGAGGTACATCCCGCCTGGAGGGCCTACAGTCAGAAAAC
CATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAAGTCACCATGCAGCTGCAAGACGTTGGCGGTTGCTAATGAGCGCGCTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAA
AACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCG
CCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTT
CCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTT
CGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTA
AAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAA
ATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTT
TTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTC
CAATGATGAGCACTTTTCGACCGAATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGA
TCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTGTCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCAC
CGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCA
CAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTACGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCA
TGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTAT
TGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAG
GTGCTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAATTTTTTTAAGGCAGT
TATTGGTGCCCTTAAACGCCTGGTTGCTACGCCTGAATAAGTGATAATAAGCGGATGAATGGCAGAAATTCGAAAGCAAATTCGACCCGGTCGTCGGTTCAGGGCAGGGTCGTTAAATAGCCGCTTATG
TCTATTGCTGGTTTACCGGTTTATTGACTACCGGAAGCAGTGTGACCGTGTGCTTCTCAAATGCCTGAGGCCAGTTTGCTCAGGCTCTCCCCGTGGAGGTAATAATTGACGATATGATCCTTTTTTTCT
GATCAAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTT
TTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATA
CTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGT
TGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAA
GCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCC
ACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTG
CGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAC
CGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA

Fig. 26B